Variability of Hormonal Stress Markers and Stress Responses in a Large Cross-Sectional Sample of Elephant Seals

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LONG-TERM GOALS

The Office of Naval Research recently funded a multi-year, collaborative research project to investigate baseline hormone variations in northern elephant seals (*Mirounga angustirostris*) and studies of the hypothalamus-pituitary-adrenal (HPA) and hypothalamus-pituitary-thyroid (HPT) axes (grant # N000141110434). These investigations described natural variation in corticosteroid, thyroid, and catecholamine hormones in northern elephant seals across multiple matrices, and assessed the sensitivity of HPA and HPT axes. Using the large sample bank from this project a number of opportunities to better understand the stress response in pinnipeds, and marine mammals overall, are available. These include the relatively low cost ability to better understand the role the binding globulins play in modulating the bioavailability of stress hormones and the mechanisms by which stress hormones interact with energy metabolism, salt balance, reproductive and immune systems. These research goals will increase our understanding of when stress has biologically significant effects on animals and identify potential mediators and mechanisms of those effects.

OBJECTIVES

The objectives of this effort are to: 1) determine the bioavailability of cortisol in serum samples and variability of cortisol binding globulin (CBG) with life-history stage and gender in elephant seals; 2) determine the impact of variation in baseline cortisol on thyroid hormones and function in elephant seals; 3) determine the impact of baseline variation in aldosterone on electrolyte balance in elephant seals; 4) determine the natural life-history variation in sex hormones for both genders and impact of variation in baseline cortisol on reproductive hormones; and 5) examine the ecoimmunology of breeding and molting in elephant seals and determine the impact ofvariation in cortisol on immune function.

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APPROACH

Task 1 – Corticosteroid Binding Globulin (CBG)

Most circulating cortisol is bound to carrier proteins—primarily corticosteroid binding globulin (CBG). Only free (unbound) cortisol is thought to be biologically active and capable of interacting with target tissue receptors. Therefore, the metabolic influence of cortisol on target tissues is mediated by carrier protein expression, primarily that of CBG. The exact role of CBG in stress responses is not well understood. It may enhance transport and delivery of corticosteroids to specific target tissues or, conversely, act as a buffer mediating alterations in circulating corticosteroids. Nevertheless, assessing the level of circulating CBG and its variability over time permits far greater understanding of circulating corticosteroid levels and their magnitude of effect on target tissues. CBG may, in fact, be an accurate marker of long term stress as it does not seem to vary with acute stress, like capture shock, in some species. Ultimately, CBG levels may directly influence the bioavailability of cortisol and modulate its biological effects. Variation in CBG may help explain some of the several orders of magnitude variation in cortisol levels observed among marine mammal species. We have validated a radioimmunoassay (RIA) for CBG in elephant seals (Immuno-Biological Laboratories, Inc, USA). We are in the process of developing a H^3tracer based measure of total plasma cortisol binding capacity. We had the opportunity to interact with Rudy Boonstra at the ONR program review who offered his collaboration and expertise in this task. Our goal is to calculate the binding affinity of CGB for elephant seals and measure cortisol binding capacity and thereby free cortisol in the entire elephant seal sample bank.

Task 2 – Impact of cortisol on thyroid function.

Thyroid hormones (thyroxine, T4; and triiodothyronine, T3) regulate many metabolic pathways, upregulating mitochondrial proliferation, facilitating erythropoesis and strongly influencing whole-animal metabolic rate. Variation in thyroid hormones directly impact energy expenditure as well as reproductive behavior and effort in elephant seals. Most thyroid hormone is released from the thyroid gland as T4. At target tissues, deiodinases (D1 and D2) convert T4 into the more biologically active T3. A third deiodinase (D3) inactivates T3 but may also convert T4 into an inactive form—reverse T3 (rT3). The rT3 binds to T3 receptors without upregulating gene expression, thus blocking most thyroid hormone action. Many stressors decrease D1 activity, including food limitation, illness, and high cortisol and catecholamine levels. The stress-induced decrease in D1 activity causes increased rT3, which likely leads to reductions in metabolic rate by blocking T3 receptors. The influence of thyroid hormones on the stress response is, therefore, significantly influenced by rT3 concentration. Preliminary analysis suggests strong association of cortisol and thyroid levels in elephant seals (Figures 2 and 3). We have validated an RIA for rT3 in elephant seals (Alpco Immunoassays, Salem, NH) and found high levels of rT3. We propose to measure rT3 in the entire elephant seal blood sample bank to better understand the interaction of cortisol with thyroid hormones. High levels of rT3 in bottlenose dolphins (ONR N000141110436) suggest these findings may have broad implications for marine mammal species.

Task 3 – Impact of aldosterone variability on osmolality

Work on the Parent Project and a parallel project on bottlenose dolphins has shown the importance of aldosterone as a stress hormone in marine mammals. Aldosterone covaries with cortisol in many groups (Figure 4) and ACTH challenges in the Parent Project have shown that ACTH is a more potent secretagogue for aldosterone than cortisol. Aldosterone is an important osmoregulatory hormone regulating salt and water balance in mammals and its function is particularly important in marine mammals (Ortiz et al. 2006). We propose to use standard laboratory techniques to measure Na^+, K^+

and osmolality in the entire collection of elephant seal blood samples to better understand the interaction of aldosterone with salt and water balance. Similar findings in dolphins suggest this stress hormone may be a key difference to stress responses in terrestrial mammals and of critical importance given the hyperosmotic environment of marine mammals.

Task 4 - Impact of stress hormone variability on sex hormones
In terms of biologically significant impacts of stress responses in marine mammals, one key factor is the interaction of stress hormones with reproductive systems. Cortisol may directly impact the gonadal axis of many species (reviewed in Moore and Evans 1999) and alter or suppress reproductive behavior. Work on the Parent Project has shown that cortisol levels vary with foraging success and allow prediction of natality in female northern elphant seals, but the underlying mechanisms are not understood. We propose to measure several key sex hormones (progesterone, estradiol and prolactin in females, testosterone in males, and dehydroxyepiandosterone (DHEA) in both sexes) for the entire collection of elephant seal samples and compare these data to the baseline variation in stress hormones. We have validated assays for all of the hormones (DPC, Seimens, Los Angeles, CA; Kelso et al. 2012).

Task 5 - Impact of stress hormone variability in immune function.
The most likely mechanism by which stress impacts may have biologically significant effects on marine mammals is through impacts on the immune system, which alter susceptibility to disease and health. We propose to measure several key markers of immune status and systemic inflammation in elephant seals. Interleukin (IL)-6 and IL-1b are produced at the site of inflammation and plays a key role in the acute phase response as defined by a variety of clinical and biological features. IL-6 is the chief stimulator of the production of acute-phase proteins in response to injury, inflammation or illness (Gauldie at al. 1987). IL-1b is an important mediator of the inflammatory response and directly regulates production of haptoglobin. Tumor necrosis factor (TNF) α is a cytokine that regulates acute phase proteins and is primarily involved in the regulation of immune cells. C-reactive protein (CRP) is an acute phase protein that serves as a marker for systemic inflammation. Immunoglobulin E (IgE) is a mammalian immunoglobulin that regulates immunity to parasitic infections and plays a role in allergic reactions. IgG is the main antibody isotype produced in response to infections. IgM is the first antibody to appear in reponse to initial exposure to an antigen. We have validated assays for IL-1b, IL-6, TNFα, CRP, IgE, IgG, IgM and haptoglobin in elephant seals (Cayman Chemical, Ann Arbor, MI; DPC/Seimens, Los Angeles, CA; Thermo-scientific, Rockford, IL). We propose to measure these markers in the entire pool of elephant seal blood samples to better understand the interaction of stress with the immune response in marine mammals.

WORK COMPLETED

Task 1 – *Corticosteroid Binding Globulin (CBG)*.
We validated and used a RIA to measure CBG in 80 adult male, 40 adult female and 40 juvenile samples. We are coordinating with Rudy Boonstra to complete measurments of binding affinity and total binding capacity in elephant seals as well as to provide samples for many other pinniped species.

Task 2 – Cortisol impacts on thyroid function.
We have completed measurments of rT3 in 116 adult male, 20 adult female and 58 juvenile samples.

Task 3 – Impact of aldosterone variability on osmolality
We have developed a autoanalyzer based system for precisely measuring Na^+, K^+, and total osmolality in serum samples. We are beginning to make these measurments in the elephant seal sample bank.

Task 4 - Impact of stress hormone variability on sex hormones
We have focused our initial efforts on reproductive impacts in females. We have validated assays for progesterone, estradiol, DHEA, and FSH. We have completed progesterone measurments in 585 adult female samples. We have completed estradiol measurments in 175 adult female samples. We have completed DHEA measurments in 165 adult female samples.

Task 5 - Impact of stress hormone variability in immune function.
We have focused our initial efforts on immune impacts in adult animals. We have measured Il-6, Il-1b, haptoglobin, IgG, IgM, and IgE in 198 adult female samples and 98 adult male samples.

RESULTS

Task 1 - Preliminary analysis shows that CBG tracks changes in cortisol during breeding but not during the molt. This suggests cortisol bioavailability is regulated according to life history stage and the CBG is a key determinant of cortisol impacts on physiology.

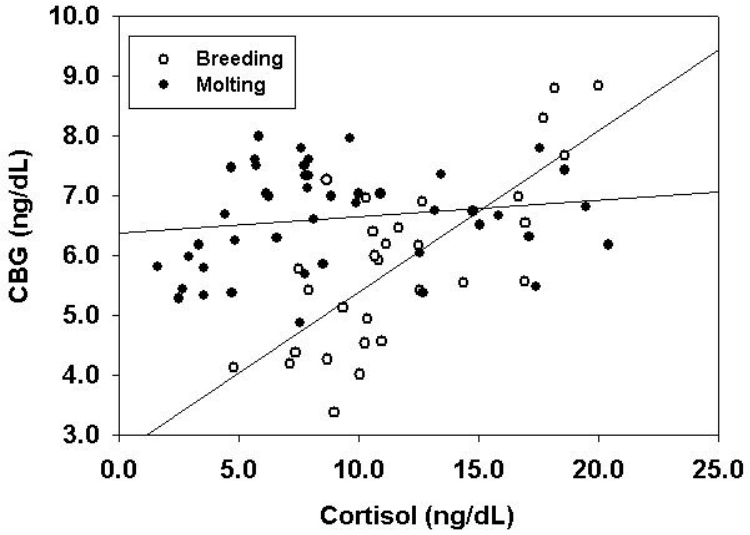

Figure 1. Changes in CBG levels with cortisol in breeding and molting adult male elephant seals

Task 2 - Preliminary analysis suggests unsually high rT3 levels in elephant seals and a strong positive relationship between cortisol and rT3 levels.

Task 3 – Analysis of samples is ongoing.

Task 4 – Analysis of the entire adult female sample bank shows that progesterone levels are low during breeding but increase dramatically during the molt. Values are highest at the end of the molt when implantation is thought to occur, suggesting an important role for progesterone in creating the uterine conditions necessary for implantation. Despite this drmatic increase there was wide individual variation in progesterone at this time. This variation was significantly negatively related to cortisol levels suggesting that suppression of prgesteone by cortisol may be he mechanism underlying cortisol impacts on natality seen in the previous project.

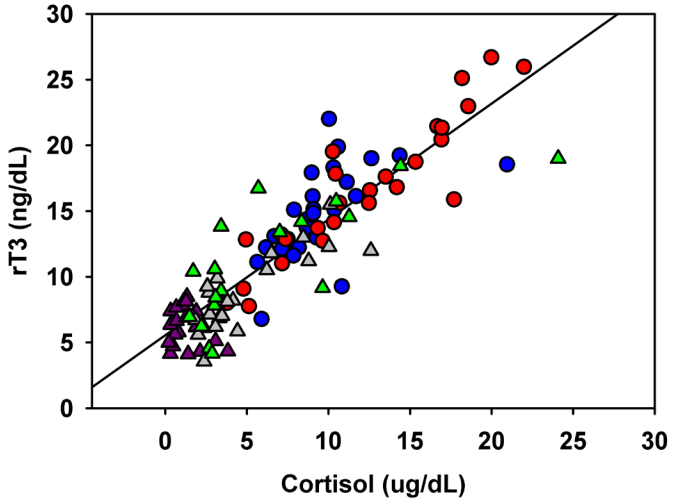

Figure 2. Relationship of rT3 to cortisol in breeding and molting male adult elephant seals.

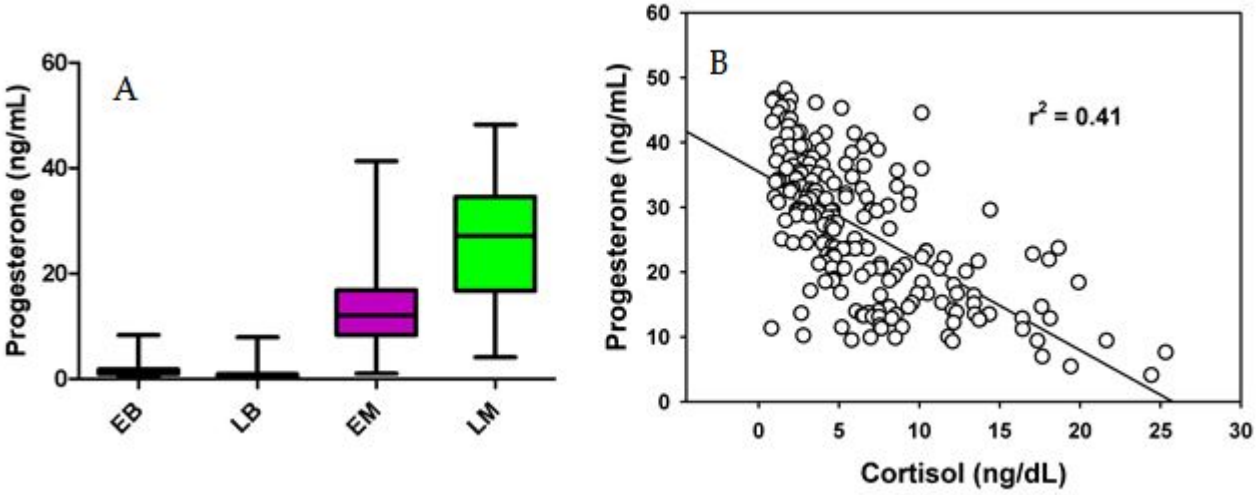

Figure 3. A: Changes in serum progesterone during the breeding and molt haul-outs in female elephant seals. EB = Early breeding, LB = late breeding, EM = early molt, LM = late molt. B: Relationship of serum progesterone to serum cortisol in late molt females.

Task 5 – In adult females, all immune markers varied significantly with life history stage. In general, immune responses were greater and more varied during the breeding haul-out, particularly in samples closest to parturition. With one exception immune markers were not associated with plasma cortisol levels. The exception to this pattern was the immunoglobulin IgE, a marker of immune response to parasite infection. IgE was highest after the post-breeding foraging trip and exhibited a significant negative association with cortisol across all life history stages. This association was strongest directly after foraging trips. IgE also declined significantly over the four study years, despite similar foraging success, suggesting declining parasite exposure or response across the study period. These data suggest that breeding carries an immune cost in female northern elephant seals, but that elevation of cortisol in association with breeding fasts does not suppress immune function. In contrast, immune response to parasites may be influenced by variation in plasma cortisol during foraging.

IMPACT/APPLICATIONS

The ability to estimate the effects to marine mammals and marine mammal populations resulting from anthropogenic sound exposure directly affects the United States Navy's ability to comply with Federal regulations regarding environmental impacts. It is critical that as information on stress related hormones are acquired as part of acoustic impact studies, that the Navy understand the biological impacts of measured variation. The additional characterization of hormones and physiological responses under widely varying baseline and simulated stress conditions, as described in the current proposal, provides a mechanism by which to better detect the presence and magnitude of the physiological responses of marine mammals exposed to anthropogenic sound. In accordance with National Research Council recommendations (2005), this work, together with the Parent Project, will establish baseline and activated levels for putative stress markers in marine mammals. This proposal makes the most effective implementation of ONR's investment strategy for evaluating stress in marine mammals by minimizing costs and maximizing the return on investment using the unique database generated by the Parent Project. Specifically, understanding the mechanisms by which stress impacts physiology will allow better determination of whether or not stress has biologically significant impacts on marine mammals.

RELATED PROJECTS

Project: Variability of Hormonal Stress Markers Collected from a Managed Dolphin Population
PI: Dorian Houser
This project examines variation in stress hormone markers across several matrices in a captive dolphin population, allowing intensive longitudinal sampling in contrast to the broad, cross-sectional sampling of our study.

Stress Hormones and their regulation in a captive dolphin population
PI: Cory Champagne
This project examines roles of CBG and rT3 in the sister study on the Navy captive bottlenose dolphin population.

PUBLICATIONS

IN PREPARATION

Peck, H.E. D.P. Costa, and D.E. Crocker. Immune response varies with life-history stage in female northern elephant seals (targeted for submission to Conservation Physiology).